2005

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Arch. Environ. Contam. Toxicol. 49, 76–88 (2005)
DOI: 10.1007/s00244-004-0172-3

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Contamination
and Toxicology

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Received: 28 July 2004 / Accepted: 18 January 2005

Abstract. The Tri-States Mining District (TSMD) of Missouri (MO), Kansas (KS), and Oklahoma (OK), USA, was mined for lead (Pb) and zinc (Zn) for more than a century. Mining ceased more than 30 years ago, but wastes remain widely distributed in the region, and there is evidence of surface- and groundwater contamination in the Spring River-Neosho River (SR-NR) system of northeastern OK. In October 2001, we collected a total of 74 fish from six locations in the SR-NR system that included common carp (*Cyprinus carpio*), channel- and flathead catfish (*Ictalurus punctatus* and *Pylodictis olivaris*), largemouth- and spotted bass (*Micropterus salmoides* and *Micropterus punctulatus*), and white crappie (*Pomoxis annularis*). We obtained additional fish from locations in MO that included three reference sites and one site that served as a ''positive control'' (heavily contaminated by Pb). Blood, carcass (headed, eviscerated, and scaled) and liver (carp only) samples were analyzed for cadmium (Cd), Pb, and Zn. Our objectives were to assess the degree to which fish from the OK portion of the SR-NR system are contaminated by these elements and to evaluate fish blood sampling for biomonitoring. Concentrations of Cd and Pb in carp and catfish from OK sites were elevated and Pb concentrations of some approached those of the highly contaminated site in MO, but concentrations in bass and crappie were relatively low. For Zn, correlations were weak among concentrations in the three tissues and none of the samples appeared to reflect site contamination. Variability was high for Cd in all three tissues of carp; differences between sites were statistically significant ($p < 0.05$) only for blood even though mean liver concentrations were at least 100-fold greater than those in blood. Blood concentrations of Cd and Pb were positively correlated ($r^2 = 0.49$ to 0.84) with the concentration of the same element in carp and catfish carcasses or in carp livers, and the corresponding multiple regression models were highly significant ($p \leq 0.001$). Our data indicate that potentially nonlethal blood sampling can be useful for monitoring of selected metals in carp, catfish, and perhaps other fishes.

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The Tri-States Mining District (TSMD) of Missouri (MO), Kansas (KS), and Oklahoma (OK), USA, was extensively mined for zinc (Zn), lead (Pb), and other metals from the mid-1800s through the 1950s. In Jasper Co., MO, Cherokee Co., KS, and Ottawa Co., OK, there are unremediated sites contaminated to varying degrees by historical mining and ore-processing activities. Metals from these sites have contaminated surface waters, groundwater, stream sediments, and biota in parts of the Spring River (SR) and Neosho River (NR) and their tributaries (Allen and Wilson 1992; Barks 1977; Czarneski 1985; Davis and Schumacher 1992; Neuberger *et al*. 1990; Spruill 1987; Wildhaber *et al*. 2000; Yoo and Janz 2003). Relative to the MO and KS sections of the SR-NR system, study of aquatic resource contamination in OK has been limited.

There are many approaches for the biomonitoring of elemental contaminants in aquatic systems (Breckenridge *et al*. 2002), and these differ according to the program or study goals (*eg*, assessing risk to humans, piscivorous wildlife, or aquatic organisms), state or regional resource priorities, and the metals or metalloids of concern. Fish and mussels or other bivalves are among the most commonly sampled aquatic organisms of large-scale metal contaminant monitoring programs (Schmitt and Brumbaugh 1990, Crawford and Luoma 1993). Bivalves are useful for biomonitoring because they are sessile and are generally superior accumulators of many waterborne metals. However, in most inland waters, fish are sampled more frequently because bivalves are not plentiful or widespread enough for practical use. To protect human health, monitoring contaminants in the edible muscle tissue of fish is often the highest priority. Conversely, monitoring contaminants in fish for assessing the risk to fish and wildlife often involves whole-body sampling, but specific protocols may also include the liver, kidney, blood, brain, or gonad (Breckenridge *et al*. 2002).

Expansion of federal and state biomonitoring efforts for addressing both human and wildlife health has resulted in the removal of increasing numbers of adult fish from inland

waters. Accordingly, the application of a suitable nonlethal measurement approach (*eg*, blood sampling) could reduce the impact of biomonitoring efforts on freshwater fish populations. Blood, urine, hair, or fingernails are typically sampled as a nonlethal means of detecting human exposure to heavy metals (Hill *et al*. 1988). Blood has occasionally been sampled for avian monitoring, particularly for Pb, mercury (Hg), or selenium exposure (Dieter 1979; Henny *et al*. 1994, 2000; Flint *et al*. 1997; Franson *et al*. 1999; Santolo and Yamamoto 1999). Fish blood has also been sampled to evaluate Pb exposure (Hodson 1976; Hodson *et al*. 1977, 1984; Haux and Larsson 1982; Haux *et al*. 1986; Dwyer *et al*. 1988; Johansson-Sjobeck and Larsson 1979; Nakagawa *et al*. 1995; Schmitt *et al*. 1984, 1993) but seldom for other metals. As is true in the blood of humans and birds, Pb in fish blood concentrates in erythrocytes and can inhibit enzymes involved with heme synthesis (Finelli 1977). Thus, the concentration of Pb in blood can be linked directly to biochemical and potentially higher-level biological effects.

Two aspects of blood sampling may have historically hindered its selection for contaminant measurement in fish. First, it can be difficult to obtain a sufficient quantity from fish for analyzing multiple contaminants and second, the concentration of some metals may be too low for routine analysis when compared to other tissues such as the liver or kidney. However, the recent maturation of inductively coupled plasma mass spectrometry (ICP-MS), a highly sensitive and multielement capable method, has greatly reduced both the sample size and analyte concentration needed for routine analysis. Also, improvements in ultratrace field and laboratory procedures have allowed for more reliable measurements at low concentrations.

The two primary objectives of this paper are (1) to document and contrast metals concentrations in several species of fish from the OK portion of the TSMD, and (2) to compare metal concentrations in blood with those in liver and carcass as a preliminary evaluation of the potential for blood sampling for monitoring metal concentrations in commonly sampled freshwater fishes. The biochemical effects of metals exposure on these fish are addressed elsewhere (Schmitt *et al*. 2005).

Materials and Methods

Collection Sites, Species, and Field Procedures

Sampling sites (Figure 1, Table 1) were selected to bracket the range of contaminant conditions expected in the TSMD-affected portions of the SR and NR in northeastern OK. The six OK sites included three on the SR (sites 1, 2, 4) and three on the NR (sites 3, 5, 6). Site 5 was within Grand Lake of the Cherokees (Grand Lake), and site 6 was in the lower reach of Tar Creek (TC). Mining-derived contaminants enter the SR from discrete and dispersed sites in MO, KS, and OK (Figure 1). Metals in the NR originate primarily from tailings and other sources in the watershed of TC, which enters the NR east of Miami, OK. Lesser amounts of metals originate near the headwaters of Elm Creek, which joins the NR west of Miami. Site 3 was upstream of known TSMD pollution sources, and fish collected there were expected to reflect near-reference conditions for the region, assuming minimal fish migration influences.

Four specimens of each of three primary species were targeted at each OK site: common carp (*Cyprinus carpio*, henceforth carp), largemouth bass (*Micropterus salmoides*), and channel catfish (*Ictalurus punctatus*). Carp were obtainable at all OK sites, but substitutes were needed at some locations for the other two target species. These included spotted bass (*Micropterus punctulatus*), white crappie (*Pomoxis annularis*, henceforth crappie), or both for largemouth bass, and flathead catfish (*Pylodictis olivaris*) for channel catfish. All fish from OK sites were collected by electrofishing in October 2001. Additional specimens were collected from sites in MO (Table 1) that included reference fish from three uncontaminated sites and ''positive control'' fish from the Old Lead Belt, where contamination is well documented (Schmitt *et al*. 1984, 1993; Schmitt and Finger 1987; Gale *et al*. 2004). Reference fish included largemouth bass and channel catfish raised at our laboratory (REF-7) and catfish from a commercial fish farm (REF-8; Osage Catfisheries, Osage Beach, MO). Positive control fish included carp, largemouth bass, and spotted bass (catfish could not be obtained) collected by electrofishing from the Big River (BR) in St. Francois Co., MO, in early December 2001. Preliminary examination of the 2001 data indicated that none of the OK sites was a suitably clean reference, so additional carp were obtained by electrofishing in July 2003 from Long Branch Lake (LB, REF-10), a 5.7 km^2, multiuse impoundment in rural north-central MO. Concentrations of Pb and Cd in fishes from LB have historically been low (unpublished data, MO Department of Conservation), but Zn had not been previously measured.

Fish Processing

Because this investigation was part of a larger study designed to examine potential health risks to area residents who consume relatively large amounts of locally procured fish, the carcass samples were prepared in a manner consistent with local eating habits (details follow). Fish from each site were transported to a central location and held alive in ambient water for processing in a manner similar to that of Schmitt *et al*. (1999), generally within 4–12 h of capture. Each fish was placed on a measuring board covered with a clear polyethylene bag turned inside out. Blood (nominally 1 to 5 mL depending on fish species and size) was obtained by caudal venipuncture using a chilled, heparinized (6 IU/mL) disposable needle and syringe. After removing the needle, the first drop of blood was dispensed for another measurement and the next 0.2 to 0.5 mL was dispensed into a preweighed, acid-cleaned, 10-mL borosilicate glass test tube fitted with a tetrafluoroethylene (TFE)-lined polyethylene screw cap. The sample tube was immediately frozen in dry ice until it was returned to the laboratory, where it was stored frozen at –20°C.

Following blood collection, the fish was subdued with a blow to the head, weighed (g), and measured for total length (TL, mm). Scale samples (if present) were removed and placed in an envelope for age determination (Jearld 1983). The abdominal cavity of each fish was dissected open and its gender was determined by gonadal observation. For carp, a sample of liver (5–10 g) was dissected, placed in an acid-cleaned polyethylene vial, and frozen in dry ice. The fish was then prepared as if for consumption by the local residents (henceforth carcass). It was scaled (except catfish), eviscerated, headed, washed thoroughly in tap water, wrapped in aluminum foil, and frozen in dry ice. Between fish preparations, all contact surfaces were thoroughly cleaned with tap water; all dissecting instruments were washed with laboratory detergent and rinsed with tap water and acetone; also, the polyethylene bag on which the fish had been dissected was replaced. Carp from LB (REF-10) were prepared in the same way as OK fish except that carcass samples were not retained for analysis. Only blood was sampled from the laboratory-raised channel catfish (REF-7) because those fish could not be sacrificed. All samples (blood, liver, and cleaned carcass) were stored frozen at –20°C for 3–6 months until prepared for analysis.

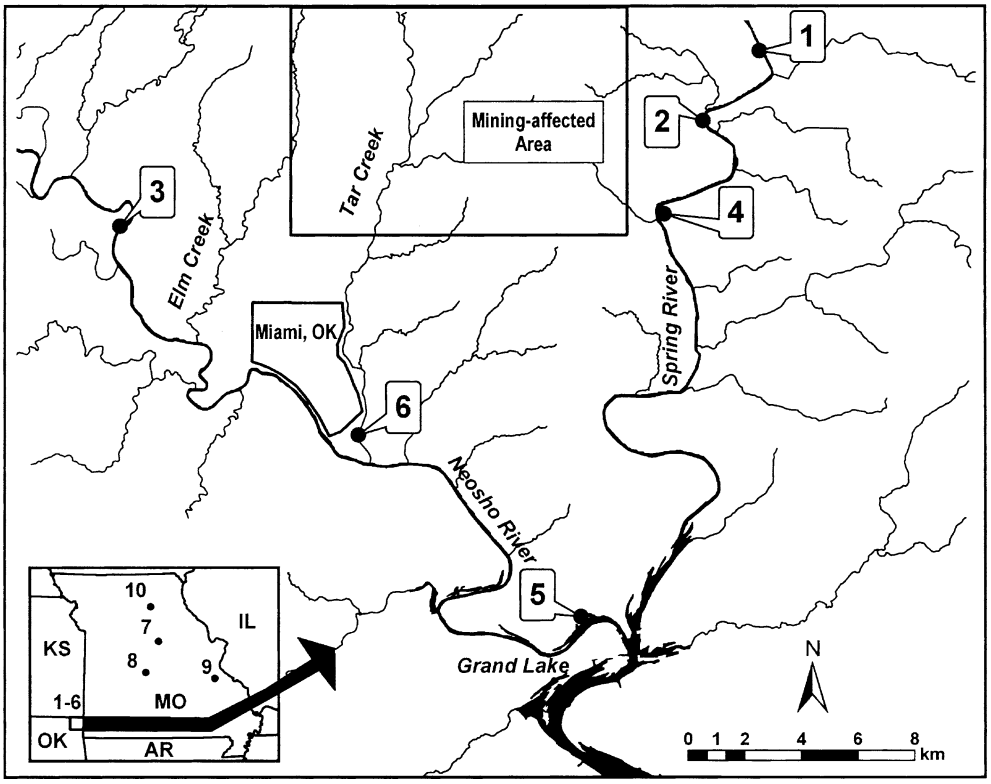

Fig. 1. Map of Northeastern Oklahoma showing the location of collection sites (1–6) on the Spring and Neosho Rivers. Also shown is the general boundary of the mining-affected area and the reference (7,8,10) and positive control (9) sites in Missouri (inset). Additional mining-affected areas are drained by the Spring River in Kansas and Missouri upstream (north) of the area shown in detail.

Sample Preparation and Digestion

In the laboratory, each frozen blood sample was weighed in its preweighed collection tube. Samples were then freeze-dried and reweighed to determine dry weight and water content. To each dried sample in its respective tube was added 1.0 mL of concentrated, subboiling distilled, HNO_3. After 1 h, the cap was tightened and the tube was placed in a mini dry-block heater set at 110°C for 30 min. Fume hood air flow provided cooling and condensation of acid vapors in the top half of each tube. Tubes were removed from the block and after cooling for 15 min, 0.2 mL of high-purity 30% (v/v) H_2O_2 was added to each. They were then resealed and returned to the block heater for 30 min. After cooling, the volume of each sample was adjusted to 10 mL with ultrapure H_2O.

Each cleaned fish carcass was briefly thawed and cut into sections with a large stainless steel knife. The sections were passed through a commercial meat grinder, mixed, then reground. An aliquot of approximately 100 g was freeze-dried and then further homogenized with a blender. A 0.25-g subsample of the dried material was digested by adding 6 mL of concentrated HNO_3 and 1 mL of 30% (v/v) high-purity H_2O_2 in a TFE-lined vessel that was sealed and heated to about 200°C in a microwave oven. Digestates were transferred to low-density polyethylene bottles and diluted to 100 mL with ultrapure H_2O. Liver samples (carp only) were freeze-dried and homogenized by pulverization with a glass rod. Subsamples (50 mg) were transferred to borosilicate glass test tubes and digested as described for the dried blood.

Instrumental Analysis

Metals were analyzed and reported as dry weight (dw) concentrations. All sample digestates were analyzed by ICP-MS with a PE/SCIEX Elan 6000 that was set up and optimized according to the manufacturer's specifications. Samples were automatically delivered to the instrument by means of a software-controlled CETAC ASX-500/ADX-100 autosampler and autodiluter system (May *et al.* 1997). All digestates were analyzed with 10-fold autodilution. The ICP-MS quantitative method was programmed to determine the following masses: ^{66}Zn and ^{68}Zn, ^{111}Cd and ^{114}Cd, and Pb as the sum of three masses (^{206} Pb + ^{207}Pb + ^{208}Pb). The internal standards were Sc (10 ppb), Rh (10 ppb), and Bi (10 ppb), which were metered into the sample line via peristaltic pump. Calibration standards for analysis were 50, 150, and 300 ng/mL for Zn; and 1.5, 3.0, and 6.0 ng/mL for Cd and Pb. During analysis, any sample digestate with a concentration exceeding the upper calibration standard for any element was automatically diluted an additional 10-fold in a serial fashion until all concentrations were within the range of the calibration standards.

For each group of samples analyzed, quality control measures incorporated at the digestion stage included blanks, certified reference materials (fish tissue, liver, or blood), replicate samples, and fortified samples (spikes). The ICP-MS quality control included periodic analyses of calibration check and laboratory control solutions, duplicate digestate analysis, analysis spikes, and interference checks (dilution percent difference and a synthetic interference solution). Except for one low recovery for Pb (53%) and one for Zn (67%), recoveries ranged from 84% to 100% for the three elements in the various certified reference tissues of blood (n = 12), fish muscle (n = 6), and liver tissue (n = 6). Recoveries from method spikes ranged from 92% to 106% for fish carcasses, 100% to 120% for blood, and 89% to 112% for carp livers. Percent relative standard deviations (%RSDs) from triplicate digestions and analyses were $\leq$ 12% for fish carcasses and $\leq$ 13% for carp livers. Duplicate collection, digestion, and analysis of fish blood samples yielded relative percent differences (RPDs) of $\leq$ 24%. Blank equivalent concentrations (BECs) of digestion blanks were all near or less than the corresponding method detection limit (MDL). The MDL was estimated based on three times the standard deviation of the three digestion blanks prepared with each group of samples. The range of MDLs in µg/g dw for carcass samples were Zn, 0.5–1; Cd, 0.008–0.03;

Table 1. Collection site locations and dates

Site no.	Water body and location	County (State)	Date	Latitude, Longitude[a]
1	Spring R at KS/OK line	Ottawa (OK)	10/15/01	36°59′50.5″N, 94°42′37.4″W
2	Spring R at Blue Hole	Ottawa (OK)	10/15/01	36°57′41.0″N, 94°43′20.6″W
3	Neosho R at Stepps Ford Bridge	Ottawa (OK)	10/16/01	36°53′25.0″N, 94°55′38.5″W
4	Spring R at Promenade Bridge	Ottawa (OK)	10/16/01	36°56′01.1″N, 94°44′40.9″W
5	Neosho R at Twin Bridges (Grand Lake)	Ottawa (OK)	10/16/01	36°47′56.0″N, 94°45′18.5″W
6	Tar Creek at Neosho R	Ottawa (OK)	10/17/01	36°51′25.7″N, 94°51′39.2″W
7	USGS-CERC (reference)	Boone (MO)	10/22/01	38°54′41.5″N, 92°16′58.0″W
8	Osage Catfisheries (reference)	Camden (MO)	10/23/01	38°07′38.9″N, 92°40′54.5″W
9	Big R @ St. Francois St. Park (pos. control)	St. Francois (MO)	12/07/01	37°57′23.1″N, 90°32′29.5″W
10	Long Branch Lake (reference)	Macon (MO)	07/10/03	39°46′00.0″N, 92°31′0.00″W

[a] From GPS, datum = WGS84.

and Pb, 0.01–0.24. Similarly, the range of MDLs (μg/g dw) for blood were Zn, 0.3–5; Cd, 0.002–0.009; Pb, 0.004–0.02; and for liver: Zn, 0.6–7; Cd, 0.002–0.005; and Pb, 0.004–0.006.

Data Set and Statistical Analyses

Total length, weight, and age data are summarized in Table 2. Carp and channel catfish were obtained from all six OK sites and two MO reference sites. Largemouth bass were obtained from three OK sites, the BR, and our laboratory. Spotted bass were obtained from three OK sites and the BR, but none was obtained from reference sites. Crappie were obtained from four OK sites only. For each variable, species-station arithmetic means and standard errors were computed and tabulated, as were summary statistics per species. All data representing elemental concentrations were $\log_{10}$-transformed before statistical analysis to control heterogeneity of variance. One-half of the MDL value was substituted for censored values (i.e., those <MDL) for all computations. A preliminary analysis of variance (ANOVA) was conducted for each variable to determine the influence of such factors as species, gender, size, age, and collection site on all measured variables. Based on this analysis, additional ANOVAs were conducted separately for each species. In these latter analyses, a one-way ANOVA in which site was considered a fixed effect was used to test for differences among collection sites. Differences among individual sites were tested with Fisher's protected LSD. Relations between and among groups of variables (transformed as described previously) were examined by using Pierson correlation coefficients and multiple linear regression. In the latter, the forward selection method was used and variables were allowed into the model only if they significantly reduced ($p < 0.05$) the unexplained sum-of-squares after other factors already included had been accounted for (i.e., the Type-II sums-of-squares were used). Closely related species (catfishes, *Micropterus* spp.) were combined for these analyses. All statistical tests were conducted using Release 8.2 of the Statistical Analysis System (SAS Institute 1999).

Results and Discussion

Carcass and Blood Moisture Content

Moisture content of fish blood and carcass was determined to allow comparison of our dw determinations to liquid (specific gravity of blood is about 1.05 g/mL) or wet weight concentrations of other studies. The mean ± standard error of blood percent moisture for each species were as follows: carp, 85.2 ± 0.3 ($n = 30$); channel catfish, 86.3 ± 0.3 ($n = 32$); flathead catfish, 85.9 ± 0.4 ($n = 4$); largemouth bass, 86.0 ± 0.4 ($n = 21$); spotted bass, 84.6 ± 0.4 ($n = 9$); and crappie, 84.9 ± 0.5 ($n = 12$). The mean ± standard error of carcass moisture for each species were as follows: carp, 75.4 ± 0.4 ($n = 25$); channel catfish, 77.4 ± 0.4 ($n = 32$); flathead catfish, 77.2 ± 0.7 ($n = 4$); largemouth bass, 77.5 ± 0.4 ($n = 21$); spotted bass, 77.1 ± 0.4 ($n = 9$); and crappie, 75.9 ± 0.4 ($n = 12$). Considering that carcass samples were headed, scaled, and eviscerated (essentially fillets plus skin and some fins and bones), moisture content of about 77% for most of these samples is consistent with other reports of roughly 75% moisture for whole fish (Schmitt and Brumbaugh 1990) and 80% for fillet (Brumbaugh *et al.* 2001).

Blood Metals Concentrations

Pb. Blood Pb concentrations differed significantly among species and sites but no other factors (size, age, gender) or interactions were significant (Table 3). Fish from the BR had by far the greatest Pb concentrations. Blood Pb concentrations among fish from the OK sites were generally greatest in carp, intermediate in catfish and bass, and lowest in crappie (Table 4). Carp, catfish, and largemouth bass from one or more of the OK sites had significantly greater blood Pb concentrations than the reference fish. Reference crappie and spotted bass were not obtained, but there were significant differences between some OK sites for blood Pb concentrations of those species. Interestingly, reference channel catfish that were reared in tanks at our laboratory had significantly lower blood Pb concentrations than those raised commercially in ponds, perhaps because the tank-reared fish could not interact with sediments.

Cd. Blood Cd concentrations differed significantly among species and sites, but no other factors or interactions were statistically significant (Table 3). For the OK sites, concentrations were generally greatest in carp and lowest in the centrarchids (Table 4). Blood Cd concentrations in carp from four of the six OK sites were significantly greater than in the reference fish, and in contrast with Pb, the greatest blood Cd concentration was in a carp from one of the OK sites. Notably, that fish also had one of the highest carcass and liver Cd concentrations. Blood Cd concentrations in channel catfish

Table 2. Site means for total length, weight, and age of each species collected (minimum, maximum, and standard error in parentheses)

Site no.	Species (n)	Total length (mm)	Weight (g)	Age (y)
1 (SR)	Common carp (4)	528 (496, 562, 17)	1705 (1650, 1800, 33)	2.3 (2, 3, 0.3)
	Channel catfish (5)	372 (310, 450, 23)	371 (185, 680, 82)	nm
	Flathead catfish (2)	628 (605, 651, 23)	3225 (2550, 3900, 675)	nm
	Largemouth bass (2)	282 (243, 320, 38)	559 (152, 444, 78)	1.5 (1, 2, 0.5)
2 (SR)	Common carp (4)	531 (484, 633, 35)	1782 (1199, 3100, 448)	2.5 (2, 3, 0.3)
	Channel catfish (1)	445 (—)	852 (—)	nm
	Flathead catfish (2)	498 (470, 525, 28)	1282 (1115, 1450, 168)	nm
	Spotted bass (3)	365 (320, 424, 31)	728 (500, 1137, 205)	2.7 (2, 3, 0.3)
3 (NR)	Common carp (4)	532 (498, 619, 29)	1804 (1517, 2200, 144)	2.8 (2, 4, 0.5)
	Channel catfish (4)	468 (396, 564, 39)	772 (405, 1248, 189)	nm
	White crappie (2)	260 (259, 260, 1)	266 (253, 278, 12)	2.0 (2, 2, <0.1)
4 (SR)	Common carp (3)	468 (443, 490, 14)	1364 (1220, 1509, 83)	2.0 (2, 2, <0.1)
	Channel catfish (5)	481 (359, 599, 44)	1027 (269, 1650, 272)	nm
	Spotted bass (3)	251 (219, 273, 16)	208 (150, 257, 31)	2.0 (2, 2, <0.1)
	White crappie (3)	277 (216, 315, 31)	274 (117, 388, 81)	1.7 (1, 2, 0.3)
5 (NR)	Common carp (3)	498 (475, 540, 21)	1544 (1270, 1950, 207)	3.3 (2, 6, 1.3)
	Channel catfish (2)	442 (349, 535, 93)	740 (283, 1196, 456)	nm
	Largemouth bass (2)	366 (349, 382, 16)	726 (575, 877, 151)	2.5 (2, 3, 0.5)
	Spotted bass (1)	213 (—)	117 (—)	1.0 (—)
	White crappie (4)	259 (240, 274, 7)	266 (194, 339, 31)	1.5 (1, 2, 0.3)
6 (NR)	Common carp (5)	470 (374, 620, 41)	1419 (616, 3100, 432)	2.4 (2, 3, 0.2)
	Channel catfish (3)	415 (276, 579, 88)	691 (133, 1500, 414)	nm
	Largemouth bass (4)	386 (278, 456, 39)	1013 (318, 1600, 267)	2.3 (1, 3, 0.5)
	White crappie (3)	339 (315, 352, 12)	607 (451, 739, 84)	2.3 (2, 3, 0.3)
7 (REF)	Channel catfish (3)	434 (395, 496, 19)	1033 (600, 1500, 260)	nm
	Largemouth bass (12)	333 (293, 398, 10)	461 (293, 837, 47)	2.3 (2, 3, 0.1)
8 (REF)	Channel catfish (12)	327 (289, 388, 8)	230 (13)	nm
9 (BR)	Common carp (2)	560 (550, 569, 10)	2225 (2100, 2350, 125)	2.5 (2, 3, 0.5)
	Largemouth bass (1)	208 (—)	103 (—)	1.0 (—)
	Spotted bass (2)	270 (245, 294, 24)	310 (217, 403, 93)	1.0 (1, 1, <0.1)
10 (REF)	Common carp (5)	534 (426, 630, 36)	2025 (1045, 3125, 367)	nm

nm = not measured.

differed significantly among reference and most OK sites, whereas Cd was universally low in blood of all three centrarchid species (11 of the 13 means were below the MDL).

Zn. Blood Zn concentrations differed significantly among species and sites (Table 3). Neither length nor weight was significant, but species–gender interaction was, indicating that gender differences were significant in at least one species. Blood Zn concentrations were generally greatest in catfish and lowest in crappie, but differences among species were less evident than for carcass concentrations (Table 4). Compared with Pb and Cd, blood Zn concentrations of carp were relatively uniform across the range of stations sampled. In contrast to carp, blood Zn concentrations of either channel catfish or largemouth bass differed significantly among some sites, but oddly, concentrations in catfish from the two reference sites exceeded those from two of the OK sites. There were some significant among-site differences for blood Zn concentrations of largemouth bass, but not for spotted bass or crappie. However, no reference fish of the latter two species were collected.

Carcass Metals Concentrations

Pb. Carcass Pb concentrations differed significantly among sites and species, but no other factors or interactions were significant (Table 3). Overall, carcass Pb concentrations were generally greatest for carp and lowest for the centrarchids (Table 4). Similar to the blood, by far the greatest carcass Pb concentrations were from carp and bass from the BR. Within-site variability of carcass Pb concentrations in carp was high for all sites; consequently, none of the OK sites differed significantly from one another. Variability was also comparatively high among individual catfish; nevertheless, means for four of six OK sites were significantly greater than that of the reference fish. Centrarchids from the OK sites had comparatively low carcass Pb concentrations and oddly, both the lowest and highest values were in fish from site 5.

Cd. Carcass Cd concentrations differed significantly among species, but overall differences among sites were only marginally significant, and no other factors or interactions were significant (Table 3). Carp had the greatest carcass Cd concentrations and centrarchids the least (Table 4). As with carcass Pb concentrations in carp, variability for Cd was relatively high, and there were no significant differences among sites. In contrast with carcass Pb, the highest mean carp carcass Cd concentration was not from the BR. Carcass Cd concentrations of catfish were relatively low (<0.1 μg/g dw in 34 of 36 samples), but variability was also comparatively low and some locations differed significantly. Only two centrarchid samples had carcass Cd concentrations >0.02 μg/g.

Zn. Carcass Zn concentrations differed significantly among locations and species, but no other factors or interactions were significant (Table 3). As noted for Pb and Cd,

Table 3. Results of analysis of variance, as *F*-Values, degrees of freedom (*df*), coefficients of determination (R^2), and significance levels (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$) for the factors and indicated variables

Source of Variation	*df*	Blood Pb	Carcass Pb	Liver Pb	Blood Cd	Carcass Cd	Liver Cd	Blood Zn	Carcass Zn	Liver Zn
Model	51	22.8***	6.81***	5.83***	3.37***	6.24***	4.37***	4.50***	9.37***	2.25*
Error (R^2)	51	0.96	0.87	0.85	0.76	0.86	0.81	0.81	0.90	0.69
Species (Sp)	5	29.4***	9.57***	—	4.41***	8.58***	—	6.38***	34.1***	—
Site (Si)	8	47.5***	11.1***	6.00***	3.78***	1.96*	2.45*	4.58***	5.72***	2.58*
Sp*Si	15	1.63*	0.85	—	1.38	0.80	—	2.07**	1.42	—
Gender (G)	1	0.78	0.05	0.51	0.34	1.70	0.08	2.04	1.51	7.62*
Sp*G	5	1.15	0.29	—	0.40	0.34	—	5.04**	1.52	—
Si*G	8	0.73	0.55	0.69	0.98	0.64	0.74	1.35	1.34	1.11
Sp*Si*G	7	0.70	1.16	—	0.34	0.96	—	0.75	1.37	—
Length	1	0.01	0.35	0.16	0.01	0.01	14.5***	2.79	2.40	1.19
Wt	1	2.27	0.60	0.43	0.21	0.12	14.4***	0.02	0.12	2.20
Age	1	—	—	1.81	—	—	2.13	—	—	1.13

Note: See text for statistical procedures.

carcass Zn concentrations were greatest for carp and lowest for centrarchids, but differences between centrarchid and catfish carcass concentrations for Zn were not as great for Pb and Cd (Table 4). There were no significant differences among sites for carp, but no reference fish were analyzed. In contrast, there were site differences for catfish, because carcass Zn concentrations from two OK sites (1 and 6) were significantly greater than all the rest (Table 4). However, the mean carcass Zn concentration of catfish from site 6 was influenced greatly by one sample (242 μg/g). This same fish also had an unusually high blood Zn concentration (155 μg/g), which suggests that the abnormal carcass concentration did not result from sampling contamination or analysis error. Mean carcass Zn concentrations of centrarchids were on the whole, slightly less than most of the catfish, and site means spanned a comparatively narrow range. Nevertheless, carcass Zn concentrations differed significantly among some sites in both largemouth bass and crappie. As noted for Pb, both the lowest and highest carcass Zn concentrations of centrarchids were in fish from site 5.

Liver Metals Concentrations (Carp Only)

Liver Pb concentrations in carp differed significantly among sites but no other factors or interactions were significant (Table 3). The mean liver Pb concentration of carp from the BR was significantly greater than of carp from all OK sites, but variability within each OK site was high and only two sites were significantly different from one another (Table 4). Liver Cd concentrations were at least 10-fold greater than those for Pb, but differences among sites were not statistically significant, largely because of high within-site variability. By far the greatest mean liver Cd concentration was in carp from site 3, but it was greatly influenced by one extreme value (319 μg/g). The blood Cd concentration in that same fish was also quite high, again suggesting that the variability was not caused by laboratory error. Interestingly, fish length and weight (but not age or gender) were significant factors for liver Cd concentration (Table 3), indicating that at least some of the Cd differences were related to fish size differences. For Zn, within-site variability of liver concentrations was relatively high, and there were no significant differences among sites. The only statistically significant factor or interaction among liver Zn concentrations (Table 3) was gender (females were higher overall).

Variation of Metal Concentrations Among Sites, Tissues, and Species

Presumably, the examination of metal concentrations among tissues and species is a useful means to assess relative site contamination and aquatic exposure. At each site, carp contained the greatest metal concentrations in both blood and carcass (Table 4). The lone exception was Zn in blood, which tended to be greatest in catfish. Fish from the BR (carp and bass) had by far the greatest Pb concentrations, but Cd and Zn concentrations were greatest in some of the OK fish. These findings are consistent with the fact that mining near the BR was predominantly for Pb, whereas in the TSMD the extraction of Zn was most important. Among the OK sites, mean carcass Pb concentrations were greatest in carp from sites 1, 2, and 3, but blood Pb concentrations were greatest in carp from sites 2 and 4. The greatest mean carcass Cd concentrations were in carp from sites 3 and 6, whereas blood Cd concentrations were greatest in carp from sites 1 and 3. Finally, the two greatest mean carcass Zn concentrations were in carp from sites 2 and 4, whereas the greatest liver Zn concentrations were in carp from sites 2, 5, and 10 (REF). Thus, the relative ranking among sites based on mean concentrations of each carp tissue type was not entirely consistent for the three metals and consequently, each tissue type presented a somewhat different indication of the relative metal exposure among sites. However, sites 2, 3, and 4 were generally among the highest for each tissue/element combination. Because carp are relatively mobile, variability in metal concentration patterns among the different tissues of individuals collected at each site may have resulted from differences in individual location preferences and associated feeding opportunities. Also, near-flood conditions existed in the area in the weeks before our fish collections, which may have contributed to the observed variability by causing some fish to move from their usual areas.

Table 4. Concentrations (μg/g dw)[1] of Pb, Cd, and Zn in fish tissue samples from Northeastern OK Sites (1–6) and reference (7, 8, 10) and mining (9) Sites in MO

Species, Site No.	n/*df*	Blood Pb	Carcass Pb	Liver Pb	Blood Cd	Carcass Cd	Liver Cd	Blood Zn	Carcass Zn	Liver Zn
Common carp										
1 (SR)	4	1.34 (0.22)bc	2.46 (1.19)a	0.60 (0.14)bc	0.086 (0.048)ab	0.18 (0.11)a	24.6 (13.4)a	86.6 (10.2)a	131 (16)a	707 (171)a
2 (SR)	4	1.88 (0.44)bc	2.12 (0.58)a	0.85 (0.14)bc	0.055 (0.012)ab	0.18 (0.06)a	26.3 (7.4)a	60.9 (3.7)a	192 (21)a	1950 (915)a
3 (NR)	4	0.73 (0.08)d	2.20 (1.18)a	0.41 (0.14)c	0.136 (0.085)a	0.67 (0.34)a	124. (72)a	112 (43)a	117 (12)a	791 (142)a
4 (SR)	3	2.31 (0.68)b	1.58 (0.40)a	1.06 (0.22)b	0.021 (0.010)ab	0.12 (0.02)a	12.4 (5.4)a	79.5 (14.7)a	180 (51)a	980 (526)a
5 (NR)	3	1.04 (0.33)bc	0.75 (0.42)a	0.91 (0.28)bc	0.017 (0.013)c	0.15 (0.09)a	13.8 (7.6)a	59.2 (3.2)a	108 (13)a	1130 (80)a
6 (NR)	5	1.23 (0.49)cd	1.17 (0.39)a	1.27 (0.70)bc	0.023 (0.012)bc	0.40 (0.23)a	13.5 (8.8)a	65.5 (20.2)a	140 (26)a	741 (113)a
9 (BR)	2	22.1 (2.8)a	12.8 (6.9)a	3.34 (0.52)a	0.125 (0.078)a	0.51 (0.31)a	17.9 (13.6)a	60.2 (12.8)a	103 (15)a	665 (8)a
10 (REF)	5	0.29 (0.04)e	—	0.15 (0.02)d	0.008 (0.001)c	—	4.9 (0.8)a	52.0 (2.7)a	—	1110 (236)a
ANOVA[2]	29/24									
F	7/6	16.97***	1.99	7.05**	2.96*	1.01	1.70	1.25	1.77	1.26
R^2	22/18	0.84	0.40	0.69	0.49	0.25	0.35	0.28	0.37	0.29
Channel catfish										
1 (SR)	5	1.31 (0.05)a	1.66 (0.72)a	—	0.030 (0.010)ab	0.094 (0.045)c	—	91.3 (4.7)bc	114 (6)a	—
2 (SR)	1	0.63abc	0.70ab	—	0.024abc	0.076abc	—	67.0d	64.6bc	—
3 (NR)	4	0.28 (0.09)d	<0.24 (<0.12)bc	—	0.020 (0.005)ab	0.033 (0.015)abd	—	86.8 (7.3)c	65.1 (5.1)bc	—
4 (SR)	5	0.58 (0.14)b	0.39 (0.09)b	—	0.020 (0.009)abc	0.029 (0.009)bd	—	124 (14)ab	65.3 (9.0)bc	—
5 (NR)	2	0.27 (0.09)cd	0.22 (0.18)bc	—	<0.009 (<0.006)cd	0.036 (0.026)abcd	—	107 (15)abc	45.6 (13.6)c	—
6 (NR)	3	0.57 (0.16)b	0.91 (0.65)ab	—	0.028 (0.020)abc	0.12 (0.08)ac	—	114 (21)abc	121 (61)ab	—
7 (REF)	3	0.02 (0.01)e	—	—	<0.007 (<0.004)d	—	—	106 (9)abc	—	—
8 (REF)	12	0.17 (0.01)d	0.08 (0.01)c	—	<0.007 (<0.002)d	<0.03 (<0.01)d	—	122 (4)a	54.5 (2.7)c	
ANOVA[3]	34/31									
F	7/6	37.89***	8.75***	—	6.51***	4.31**		3.97**	5.17**	—
R^2	27/25	0.91	0.68	—	0.63	0.51		0.51	0.55	—
Flathead catfish										
1 (SR)	2	0.28 (0.03)a	<0.24 (<0.17)a	—	0.026 (0.006)a	0.031 (0.001)a	—	104 (4)a	49.0 (4.5)a	—
2 (SR)	2	0.23 (0.08)a	<0.24 (<0.17)a	—	0.048 (0.033)a	0.042 (0.006)a	—	103 (16)a	48.9 (3.5)a	—
Largemouth bass										
1 (SR)	2	0.28 (0.06)b	<0.24 (<0.17)b	—	<0.009 (<0.006)a	<0.02 (<0.01)	—	73.6 (6.3)a	64.6 (1.4)a	—
5 (NR)	2	0.10 (0.02)c	0.025 (0.001)c	—	<0.009 (<0.006)a	<0.008 (<0.006)	—	67.3 (11.8)ab	30.4 (1.3)d	—
6 (NR)	4	0.25 (0.02)b	0.066 (0.008)bc	—	0.025 (0.004)a	<0.008 (<0.004)	—	83.0 (6.7)a	39.0 (2.2)c	—
7 (REF)	12	0.10 (0.01)c	0.046 (0.012)c	—	<0.007 (<0.002)a	<0.008 (<0.002)	—	53.9 (1.9)b	53.0 (1.9)b	—
9 (BR)	1	14.4a	6.76a	—	<0.007a	<0.03	—	67.2ab	52.4ab	—
ANOVA	20									
F	4	38.86***	13.40***	—	1.64	—	—	8.25***	15.22***	—
R^2	16	0.91	0.77	—	0.29	—	—	0.67	0.79	—
Spotted bass										
2 (SR)	3	0.59 (0.02)b	<0.24 (<0.14)b	—	<0.009 (<0.005)a	<0.02 (<0.01)a	—	75.7 (2.4)a	55.2 (3.4)a	—
4 (SR)	3	0.38 (0.07)c	<0.24 (<0.14)b	—	<0.009 (<0.005)a	<0.02 (<0.01)a	—	82.0 (5.3)a	61.5 (2.8)a	—
5 (NR)	1	0.23c	0.23b	—	<0.009a	0.011a	—	83.7a	80.5a	—
9 (BR)	2	8.11 (0.58)a	3.21 (0.53)a	—	0.013 (0.006)a	0.026 (0.011)a	—	79.0 (14.2)a	62.3 (7.1)a	—
ANOVA	8									
F	3	105.5***	33.21**	—	1.61	4.56	—	0.22	3.08	—
R^2	5	0.98	0.95	—	0.49	0.73	—	0.11	0.64	—
White crappie										
3 (NR)	2	0.12 (0.04)bc	<0.24 (<0.17)ab	—	<0.009 (<0.006)	<0.02 (<0.01)a	—	69.4 (7.9)a	42.1 (3.0)b	—

Table 4. Continued

Species, Site No.	n/*df*	Blood Pb	Carcass Pb	Liver Pb	Blood Cd	Carcass Cd	Liver Cd	Blood Zn	Carcass Zn	Liver Zn
4 (SR)	3	0.32 (0.05)a	0.24 (0.07)a	—	<0.009 (<0.005)	0.008 (0.004)a	—	64.9 (5.1)a	70.0 (3.1)a	—
5 (NR)	4	0.10 (0.01)c	0.070 (0.009)b	—	<0.009 (<0.004)	<0.008 (<0.004)a	—	58.7 (3.8)a	50.7 (3.0)b	—
6 (NR)	3	0.21 (0.04)ab	0.100 (0.027)b	—	<0.009 (<0.005)	<0.008 (<0.005)a	—	66.3 (5.7)a	47.0 (2.8)b	—
ANOVA	11									
F	3	9.98**	4.71*	—	—	1.95	—	1.41	10.21**	—
R^2	8	0.79	0.64	—	—	0.42	—	0.35	0.79	—

Notes: See text for statistical procedures. ANOVA statistics not reported for flathead catfish due to small n, nor for Cd in carcass of largemouth bass or Cd in blood of white crappie because all values were < MDL. Within species, element, and tissue type, values followed by the same letter are not significantly different ($p > 0.05$). Also shown are one-way ANOVA (site as fixed effect), degrees of freedom (*df*), *F*-values followed by significance level (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$), and coefficients of variation (R^2).

[1] unweighted arithmetic site means and standard errors (in parentheses).

[2] *df* for blood and liver followed by *df* for carcass (carp carcass samples were not analyzed from site 10).

[3] *df* for blood followed by *df* for carcass (channel catfish carcass samples were not analyzed from site 7).

Metal concentrations in catfish approached those measured in carp for some sites (Table 4), particularly at site 1, where catfish blood Pb and carcass Pb concentrations were by far the greatest (but no catfish were obtained from the BR). Site 1, along with site 6, also had the greatest blood Cd, carcass Cd, and carcass Zn concentrations in catfish, but site 4 had the greatest blood Zn concentrations. In bass and crappie from the OK sites, concentrations of both Pb and Cd were low compared to carp or catfish, and blood Cd and carcass Cd concentrations were generally not distinguishable from those of the reference fish. Carp and catfish may have been more elevated in these metals because when feeding, they tend to interact more directly with the sediments where metals tend to be concentrated. However, in contrast to the OK fish, bass from the BR site had Pb concentrations that were nearly as great as the carp. Perhaps the greater relative Pb exposure apparent among bass from the BR as compared with the OK sites was due to differences in factors such as route of exposure, feeding mode, or seasonal fish movement.

Metal Concentrations in OK TSMD Fish Relative to Other Central U.S. Fish

Carcasses. Comparison of our carcass data to most other studies is complicated because either boneless/skinless fillets or whole fish are usually analyzed. Concentrations of Pb, Cd, and Zn are generally greater in whole bodies or livers of fish than in fillets (Goldstein and DeWeese 1999; Gale *et al*. 2004). Compared to the axial muscle (fillet) tissue, concentrations of many metals are greater in certain internal organs (Savory *et al*. 1987) and some, including Pb, are enriched in the skin and bone (Schmitt and Finger 1987). Therefore, it is reasonable to expect a ''cleaned'' carcass (headed, scaled, and eviscerated) concentration to be less than that of a corresponding whole fish but greater than a boneless/skinless fillet, particularly for Pb. Accordingly, metals in fish from the OK portion of the NR-SR appear to be similar to those of other mining-impacted reaches of the SR. For example, Allen and Wilson (1992) reported Pb concentrations as high as 1.4 μg/g dw for whole carp, 1.8 μg/g for whole bluegill (*Lepomis macrochirus*), and 0.3 μg/g in other taxa collected in 1988 from the SR in KS. They also reported Cd concentrations as great as 0.5 μg/g dw in carp and 0.08 μg/g in other taxa and Zn as great as 500 μg/g in carp. In comparison, fish carcasses from our OK sites had mean Pb values of 1.7 μg/g for carp, 0.7 μg/g for catfish, and 0.1 μg/g for bass, whereas mean Cd values were 0.28, 0.065, and <0.02 μg/g for those same species. The maximum individual Zn concentration in carcasses from our study (249 μg/g) was only one-half that reported by Allen and Wilson (1992) for whole carp. However, the digestive tract was removed from our samples, and in carp the foregut tissue has been noted to be particularly high in Zn (Sun and Jueng 1998). Considering that Schmitt (2004) reported Zn concentrations in whole carp from many central U.S. river sites not associated with mining to be as great as 400 μg/g dw, those from the TSMD do not appear to be greatly elevated in Zn. However, because Zn is regulated by most aquatic organisms and generally does not bioaccumulate (Giesy and Wiener 1977), concentrations in fish may not necessarily reflect environ-

mental exposure. For example, Schmitt *et al*. (1984) reported that Zn concentrations in whole suckers (*Catostomidae*) varied by a factor of only two in MO streams in which sediment Zn concentrations varied by almost 10,000-fold. Harrison and Klaverkamp (1990) reported similarly small differences for Zn concentrations in either the liver or muscle tissue of suckers and no differences among northern pike (*Esox lucius*) from Canadian lakes with sediment Zn concentrations that differed by nearly 100-fold.

Carcass Pb concentrations of our OK carp (0.23–5.46 μg/g dw) were greater than whole carp concentrations from most Mississippi River Basin sites sampled by Schmitt (2004), but lower than corresponding concentrations in carp collected from either the BR (6–20 μg/g dw) or the Mississippi River downstream of a smelter complex at Herculaneum, MO (about 16 μg/g; Schmitt *et al*. 2002). We were unable to obtain catfish from the BR for comparison, but whole channel catfish from near Herculaneum (Schmitt *et al*. 2002) contained only slightly greater concentrations of Pb (5 μg/g dw) than the maximum concentrations in our OK channel catfish carcasses (4.3 μg/g). In contrast, carcass Pb concentrations were considerably lower in bass from OK (0.023–0.23 μg/g dw) than in bass from the BR (2.7–6.8 μg/g). In comparison, Gale *et al*. (2004) reported a mean Pb concentration of about 52 μg/g dw for whole sunfish collected from the BR during 1998–2000, but a mean of only about 0.7 μg/g for corresponding boneless/skinless sunfish fillets. They also reported a mean Pb concentration about 1.5 μg/g dw in fillets of suckers from the BR and a mean of about 0.2 μg/g dw for bass, but they did not report corresponding whole-fish concentrations for those species. Considering preparation methods, catfish and carp from the OK sites appear to be somewhat lower in Pb than fish from the heavily contaminated BR site, whereas Pb concentrations in bass from OK are considerably lower.

Liver (carp only). The USGS National Water-Quality Assessment Program (NAWQA) routinely measures the concentrations of metals in the livers of bottom-dwelling fishes (primarily carp) in major drainages of the United States (Crawford and Luoma 1993). The NAWQA database included a total of 162 composite carp liver samples that had been analyzed for Cd, Pb, and Zn (http://water.usgs.gov/nawqa/data, September 2004). The ranges of these liver concentrations (μg/g dw) were the following: Cd, 0.05–58.1; Pb, 0.05–5.4; and Zn, 85.3–1950, whereas geometric means were 3.06, 0.19, and 613, respectively. Mean carp liver concentrations for our most elevated OK sites were about 2–3 times greater than the NAWQA geometric mean for Zn, about 5–7 times greater for Pb, and about 9-40 times greater for Cd. Interestingly, Yoo and Janz (2003) reported liver metal concentrations for bluegill sunfish (*Lepomis macrochirus*) collected from TC, OK that were similar to concentrations in our carp livers collected from the mouth of that stream (site 6). Although no carp were obtained from any NAWQA sites specifically associated with mining, the NAWQA carp liver maximum for Pb was similar to that in carp livers from OK site 6 and the BR. Conversely, our lowest OK carp liver Pb concentrations exceeded the lowest NAWQA concentrations by about 10-fold. In contrast to Pb, our greatest OK carp liver concentrations of Cd and Zn were greater than the NAWQA maxima by 2- to 3-fold. However, as was true for Pb, our OK carp liver minima were considerably greater than the corresponding NAWQA minima for Cd and Zn. Carp livers from our MO reference site (LB) were universally lower in Cd, Pb, and Zn than those from the OK sites (Table 4), but they too were greater than the NAWQA minima. Although there are no direct inputs of pollutants to LB, a small marina and a nearby coal-fired power plant may be sources of metals.

Blood. Several of the taxa analyzed in this study have not been investigated previously with respect to blood metals. In studies with suckers from USA streams in MO, MT, and WA, blood Pb concentrations were typically about 0.4–0.6 μg/g dw (assuming 86% blood moisture) at reference sites and 5–15 μg/g at sites heavily contaminated by mining and related activities (Schmitt *et al*. 1984, 1993, 2002, Dwyer *et al*. 1988). Black redhorse (*Moxostoma duquesnii*) from Center Creek, a SR tributary in Jasper Co., MO, averaged about 1–2 μg/g (Schmitt *et al*. 1993), values that are nearly identical to Pb in blood of SR carp from our study (Table 4). In comparison, the concentration of Pb in blood of our LB reference carp (mean = 0.29 μg/g) was similar to that in suckers and other species collected from reference streams in MO by Schmitt *et al*. (1993). Thus, Pb in blood of carp from the OK sites was moderately elevated relative to reference fish. In contrast to carp, centrarchids from our OK sites had blood Pb concentrations that were generally similar to those in related species from minimally contaminated locations. Dwyer *et al*. (1988) reported that Pb averaged about 0.23 μg/g dw in blood of longear sunfish (*Lepomis megalotis*) from a relatively uncontaminated site on the BR (Irondale) in 1980, but about 3–7 μg/g at tailings-contaminated BR sites. Compared with the Irondale concentrations, our reference bass and crappie were slightly lower, our OK largemouth bass and crappie were similar, and our OK spotted bass were slightly greater.

Blood Cd concentrations in carp from the OK sites were considerably greater than those of reference fish but lower than those reported in suckers from the BR and other mining-affected streams in eastern MO, which was also true for suckers collected from Center Creek in 1989 (Schmitt *et al*. 1984, 1993, 2002). Blood Zn concentrations in carp from the OK sites were similar to those of suckers collected from Center Creek that Schmitt *et al*. (1993) reported; both were slightly greater than blood Zn reported in suckers from the most contaminated streams in eastern MO (Schmitt *et al*. 1984, 1993). Although blood Zn concentrations were greater in carp from OK sites than in LB reference carp, the differences were not statistically significant. We found no suitable blood data for Cd or Zn with which to compare our values for bass and crappie.

Correlations Among Blood, Liver, and Carcass Concentrations

As anticipated, many of the variables measured in this study were correlated, and strong positive correlations generally characterized relations between Pb or Cd concentrations of the same metal in different tissues. Comparison of such relationships can be useful for assessing the utility of analyzing specific tissues for these metals. Across all taxa, $\log_{10}$-

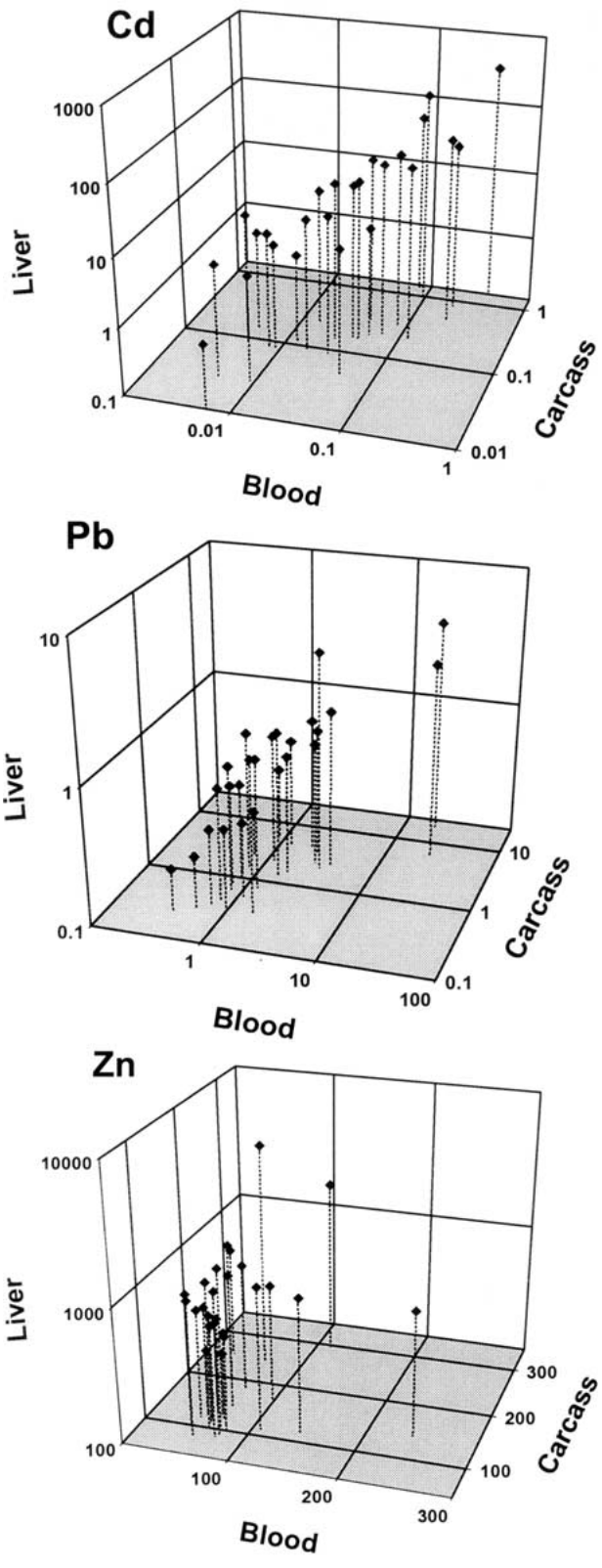

Fig. 2. Concentrations (μg/g dw) of Cd, Pb, or Zn in blood, liver, and cleaned carcass of carp from this study ($n = 30$).

transformed blood Pb and carcass Pb concentrations were highly correlated ($r = 0.88$, $p < 0.001$), as were blood Pb and liver Pb concentrations in carp ($r = 0.88$, $p < 0.001$). Also significant, but not as strong was the correlation between carcass Pb and liver Pb concentrations in carp ($r = 0.55$, $p < 0.01$). Correlations between metal concentrations in these matrices were also fairly strong for Cd ($r = 0.83$ for carp blood and liver; $r = 0.78$ for carp blood and carcass), but not for Zn ($r \leq 0.26$). These data for carp, illustrated in Figure 2, suggest that blood was comparable to either the carcass or liver as an indicator of Cd and Pb exposure. Blood and carcass concentrations were also highly correlated for Pb and Cd in channel catfish (livers not analyzed), but concentrations in centrarchids were often too low in either tissue type to adequately compare. Correlations between tissue concentrations of Pb or Cd were strong despite high within-site variability among individual fish (particularly carp). Variability was relatively low for the same measurements in the reference fish, indicating that variability among nonreference fish was due primarily to environmental factors (*e.g.* dietary and location preferences) as opposed to random sample contamination or other artifacts resulting from field or laboratory procedures.

Regression analysis statistics for liver or carcass concentrations as functions of blood concentrations and the associated statistically significant models ($p > 0.05$) are indicated for each taxon in Table 5. Some regressions were improved slightly by inclusion of blood iron concentration in the models, but these results are not presented because the improvement was minimal. For Pb, models based on blood concentrations were highly significant for all taxa except crappie ($p = 0.06$), which included only 12 observations. Models for Cd in carp or catfish blood and carcass were also highly significant, but concentrations were undetectable in most samples of bass and crappie. Excluding the limited crappie data set, coefficients of determination for Pb ranged from 0.49 (carp blood vs. carcass) to 0.86 (bass blood vs. carcass). Overall, the blood vs. liver relationship was stronger than blood vs. carcass, and Pb models were better than Cd models. Interestingly, in some instances either weight, length, or both improved the fit of the model (carcass Pb vs. blood Pb in carp, for example). However, inclusion of size metrics affected the model intercepts and could increase bias of predicted values for smaller fish.

Implications for Biomonitoring

As judged by correlations between tissues and the proportion of total variation explained by one-way ANOVA (Table 4), blood was comparable to or better than liver or carcass for assessing metal exposure in our samples. Among the elements and tissues analyzed, differences among sites were highly significant ($p < 0.01$) for all species only for Pb in blood. In carp, Cd differences among sites were only significant for blood ($p < 0.05$; Table 4), even though mean concentrations in liver were at least 100-fold greater than in blood. For Zn, differences among sites were significant for both blood and carcass in channel catfish and largemouth bass, but in some instances the reference fish had nearly the greatest concentrations (Table 4). In bass and crappie, differences among sites were significant for Pb in both blood and carcass, whereas Cd was undetectable in most samples of either tissue.

One consideration when monitoring blood is that it may poorly indicate the long-term exposure history of an organism if the exposure has been variable. This is because the biolog-

Table 5. Statistically significant ($p < 0.05$)[a] regression models for concentrations (log μg/g dw) of Cd, Pb, and Zn in fish liver or carcass (dependent variable) as a function of (a) the concentration of the same element in blood (μg/g dw, independent variable) or (b) blood concentration and any other measured variables that improved the model regression correlation (standard errors are in parentheses)

Taxon	Tissue	Element	*Df*	Model: log (tissue element concentration) =	R^2/r^2	*p*
Carp	Liver	Pb	28	−0.274(0.036) + 0.762(0.076) [log] (blood Pb)	0.78	<0.0001
		Pb	27	1.84(0.67) + 0.725(0.067) [log] (blood Pb) – 0.659(0.208) [log] wt	0.84	<0.0001
		Cd	28	2.42(0.18) + 0.841(0.107) [log] (blood Cd)	0.69	<0.0001
		Zn	—	None		
	Carcass	Pb	23	0.0005(0.078) + 0.806(0.172) [log] (blood Pb)	0.49	<0.0001
		Pb	22	−6.17(3.71) + 0.812(0.166) [log] (blood Pb) + 2.28(1.37) [log] len	0.55	0.0002
		Pb	21	−24.4(5.9) + 0.772(0.134) [log] (blood Pb) + 14.2(3.5) [log] len - 4.35(1.22) [log] wt	0.72	0.0001
		Cd	23	0.329(0.199) + 0.731(0.123) [log] (blood Cd)	0.61	<0.0001
		Zn	—	None		
Catfish	Carcass	Pb	34	−0.058(0.081) + 1.37(0.137) [log] (blood Pb)	0.75	<0.0001
		Cd	34	−0.421(0.190) + 0.565(0.092) [log] (blood Cd)	0.53	<0.0001
		Cd	33	0.656(0.392) + 0.661(0.088) [log] (blood Cd) – 0.330(0.108) [log] wt	0.63	0.0001
		Zn	—	None		
Bass	Carcass	Pb	28	−0.451(0.066) + 1.01(0.077) [log] (blood Pb)	0.86	<0.0001
		Cd	—	None		
		Zn	—	None		
Crappie	Carcass	Pb	10	−0.502(0.225) + 0.574(0.271) [log] (blood Pb)	0.31	0.06
		Cd	—	None		
		Zn	—	None		

[a] Regression model for Pb in carcass vs. Pb in blood for crappie also shown because the *p* value was near 0.05 despite only 12 observations. *wt* = fish weight (g); len = fish total length (mm).

ical ''half-life'' of most metals in blood ranges from several days to several weeks at most, whereas target tissues such as the liver, kidney, bone, or axial muscle tend to be more cumulative metal repositories (Savory *et al*. 1987). Thus, blood may be less suitable for monitoring in situations where exposure is intermittent. However, the comparability of the blood Pb concentrations we report relative to those of the previous studies cited indicates that for situations such as those described here, where exposure derives from historically deposited metals, blood concentrations accurately reflect exposure. Moreover, there are several advantages for sampling blood over other target tissues. First, an elevated metal concentration in blood may better indicate the current exposure of an organism because in many instances metal sequestration into a target tissue represents a detoxification mechanism (Martin 1987). Second, metal concentrations in blood seem to be less variable than in other tissues. Hence, fewer samples would be required to achieve the same relative precision. Third, blood is generally less costly to analyze than other tissues because collection and storage requirements are simpler and homogenization is not required. Consequently, greater sampling frequency, greater replication, or both is potentially more cost-effective for blood. Fourth, surface contamination acquired during sample preparation is presumably less likely for blood collected with a needle and syringe than for tissues obtained by dissection (Wiener 1982; Schmitt and Finger 1987). Finally and perhaps most importantly, sampling (blood) could presumably be conducted repeatedly from large fish, which are less plentiful and often more valued by recreational fishermen and fish consumers, because they would not have to be sacrificed.

Our results indicate that blood sampling is practical and can be useful for monitoring Cd and Pb in carp, catfish, and perhaps other fishes. For monitoring Zn, blood was no better or worse than carcass or liver, but none of these tissues seemed particularly useful. However, additional studies are needed to assess the potential effects on blood metal concentrations caused by varying exposure conditions, fish size, etc., and of the suitability of blood for monitoring of other important elements such as Hg (Cizdziel *et al*. 2003). One possible monitoring approach would be to sample blood primarily but periodically also collect other tissues to establish site-specific relationships of metal concentrations between tissue types. Such site-specific relationships could also provide useful information for documenting the route and duration of exposure. Regardless, potential long-term effects of blood sampling on fish health and survival must also be addressed.

Summary and Conclusion

For each element and tissue, concentrations in carp tended to be greatest, catfish were intermediate, and bass and crappie were lowest. One exception was Zn in blood, which tended to be greatest in catfish. Carp and catfish from the SR-NR system of northeastern OK were elevated in Cd and Pb, but there were no clear trends for Zn. In bass and crappie, among-site differences were statistically significant for Pb in both blood and carcass, but Cd was undetectable in most samples of either tissue. In some instances, Pb concentrations in carp and catfish from OK approached those reported for highly impacted locations in MO, but OK bass and crappie concentrations were low in comparison. Thus, Pb exposure in bass and crappie from the OK portion of the TSMD seemed to be much lower than in carp, which is in contrast to the highly contaminated BR site in MO, where Pb exposure in bass appeared to be nearly as great as in carp.

Concentrations of Cd and Pb in carp were strongly correlated among blood, carcass, and liver despite considerable within-site variability among individual fish. However, the

relative ranking among sites based on mean concentrations of each carp tissue type was not entirely consistent for all three metals. Consequently, each tissue type presented a somewhat different indication of the relative metal exposure among the OK sites. For Zn, there were minimal correlations between tissues, few differences among sites, and in some instances the greatest concentrations were in the reference fish. Within-site variability was particularly high for Cd in all three carp tissues, and differences among sites were significant ($p < 0.05$) only for blood even though mean concentrations in liver were at least 100-fold greater than in blood. Thus, although carp are commonly sampled because they are ubiquitous, presumed differences in exposure of carp in our study appeared to be masked by high variability of metal accumulation in livers and carcasses.

There are practical advantages for sampling blood in comparison to other tissues. In our study, putative exposure of carp and catfish to Cd and Pb was better indicated by concentrations in blood than by those in liver or carcass. For monitoring Zn, blood was no better or worse than the carcass or liver, but none of these tissues seemed particularly useful. However, additional studies are needed to fully assess the effects of varying exposure conditions, fish size, and other factors on the utility of blood sampling for biomonitoring of these and other trace elements, and to evaluate the long-term effects of blood sampling on fish health and survival.

Acknowledgments. This study was conducted jointly by the USGS and the U.S. Fish and Wildlife Service (USFWS) through an interagency agreement facilitated by D. Martin of the USFWS. D. Heckathorne and S. Dudding (USFWS) coordinated the collection of the fish from the sites in OK. Logistical support was provided by J. Dalgarn of the U.S. Bureau of Indian Affairs. D. Sappington of the Quapaw Tribe of Oklahoma assisted in the field. Fish from BR and LB were obtained with the assistance of M. Reed and M. Anderson of the MO Department of Conservation (MDC). Fish carcass samples were homogenized by LET Incorporated, Columbia, MO, under the supervision of E. Hinderberger. NAWQA data were summarized and provided by R. DeWeese of USGS. A. Donahue, J. Whyte, J. Arms, R. Wiedmeyer, M. Walther, S. Koppi, and J. A. Allert of CERC assisted in various aspects of the study. R. Lea of the University of Missouri-Columbia (UMC) prepared the map, and M. Ellersieck (UMC) provided statistical advice. We also thank two anonymous reviewers for helpful comments.

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